

Project 90258

## **Microbial Transformations of TRU and Mixed Wastes: Actinide Speciation and Waste Volume Reduction**

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**RESULTS TO DATE:** EMSP Project Microbial Transformation of TRU and Mixed Waste: Actinide Speciation and Waste Volume Reduction

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### Objectives

I. To characterize the biodegradation of cellulosic materials using Fourier Transform Infrared (FTIR) Spectroscopy.

II. To develop an electrochemical/spectroscopic methodology to characterize TRU waste microbial transformation

III. To develop molecular models of TRU complexes in order to understand microbial transformation

In all cases, objectives are designed to compliment the efforts from other team members, and will be periodically coordinated through the lead P.I. at Brookhaven National Laboratory (BNL), A.J. Francis.

### Summary Results to Date:

#### Results for Objective I:

Cellulosic samples were prepared 1/29/92 at BNL from various sources, including white and brown paper towel, and Kimwipes. The mixed cellulose were cut into 1 cm x 1 cm squares and transferred to glass serum bottles and various treatments were conducted: unamended (U) samples were filled with nitrogen-purged brine from G-Seep (4.1 M Na<sup>+</sup> and 5.1 Cl<sup>-</sup> with minor amounts of Mg, K, and Ca and 0.3 M sulfate (Brush, 1990)); unamended/inoculated (UI) samples were filled with bacteria-containing surface lake water, sediment, and halite from the underground at the WIPP site; amended/inoculated (AI) samples were inoculated in this fashion and amended with nutrients; and amended/inoculated/excess nitrate (AINO3) samples were inoculated with excess nitrate in the form of KNO<sub>3</sub> (5 g L<sup>-1</sup> (49.5 mM)). Further information on sample preparation is available. All samples were analyzed by Fourier transform infrared spectroscopy (FTIR) at SBU to identify any transformations in cellulosic material which may have occurred during treatment and storage.

FTIR spectra were obtained using a Nicolet 760 infrared spectrometer modified to collect data in both mid- and far infrared regions and equipped with a Continuum microscope (Spectra-Tech, Inc., Shelton, CT) to collect data in reflection mode from features down to tens of microns. Additional equipment information and details of data analysis are available. All wavenumbers used in this study and not specified to be obtained from standards were obtained from the Spectral Atlas (TM) database (Academic Press, version 1.0, 1998). In general, spectra were found to contain features associated with carbonyl from xylose or glucuronic acid, carbonyl associated with aromatic ring structures, lignin, carboxylic acid, aliphatic ligands, hydroxyl groups, and some features normally associated with alkali-treated paper. These features were identified during preliminary analysis of the data. The samples have degraded

heterogeneously as observed by the slight differences in spectra obtained from various spots on the same sample. Optical and infrared images of the samples do show more extrememphysical degradation of the samples, including some separation of individual fibers. While some chemical differences have been noted between samples, these are only on the order of differences found within a single sample. For statistical purposes, we have averaged a series of spectra obtained from the same sample. This averaging has undermined the intensity of degradation. Currently, the spectra are being analyzed individually to determine the heterogeneity in degradation. Identifying particular ligands will also assist in understanding spectra to be gathered from TRU-cellulosic material interactions.

#### Future work on Objective I:

One of the main problems in characterizing cellulosic samples is that the absorption features for carboxylic and aromatic groups fall in the region where the H-O-H angle vibration group absorption from water is intense ( $\sim 1650\text{ cm}^{-1}$ ). The presence of moisture in the samples gives rise to broad unresolved bands compromising the amount of information that can be derived from these spectra. One of the methods to overcome this problem is to expose the samples to a controlled D<sub>2</sub>O atmosphere to replace the adsorbed water molecules with deuterated water. D<sub>2</sub>O absorb at lower frequencies and hence will not compromise the effect of aliphatic and aromatic absorption features. We plan to build a system to expose cellulosic materials in controlled D<sub>2</sub>O atmosphere. The results from such experiments will be included in future reports.

#### Results for Objective II:

Establishment of electrochemical equipment and protocol for study of electron transfer kinetics associated with TRU/bacterial interactions in aqueous media.

Progress has been made in the preliminary stages of this objective, including: (a) a Gamry Femtostat (capable of accurately detecting currents in the femtoampere range) has been set up on site at BNL to perform analysis of TRU and related materials in solution; (b) a preliminary testing protocol has been established by which bacteria are to be immobilized on glassy carbon electrodes to monitor electrochemical activity in solution containing low concentrations of TRU. Glassy carbon has been shown to be an ideal surface for immobilization of bacterial material. This immobilization may be assisted using medical dialysis membrane material or other chemically inert membrane material with appropriate hole size. Open circuit potential monitoring will be conducted in specially designed cells inoculated with bacteria to analyze kinetics during metabolic processes, with and without the presence of TRU. In addition, analogs for TRU will be analyzed in equivalent electrochemical conditions at Stony Brook using in situ Raman spectroscopy to monitor transformation.

Recently, we have completed a series of experiments using in situ X-ray absorption analysis at the National Synchrotron Light Source during electrochemical polarization of solutions containing very low concentrations ( $< 5\text{ mM}$ ) of uranium-organic ligands. The success of these experiments has been the result of optimization of a custom-designed sealed cell using polished glassy carbon electrodes with data collection greatly enhanced by using a thirty element Si detector. This has made possible identification of outer sphere adsorption and inner sphere transformations of organically liganded uranium as a result of electron transfer kinetics. As far as we can tell, this is the first time such experiments have been used or have yielded useful data, and we expect this to become a model for our future work with actinides.

#### Results for Objective III:

Molecular modeling of inorganic complexes and organically-liganded TRU

Ab initio self-consistent field (SCF) and density functional theory (DFT) is being used to model Pu-inorganic and organic complexes in order to determine likely structures and predict vibrational frequencies which would be affected by transformation and speciation changes in TRU-organic and

inorganic complexes exposed to bacteria. By also determining the highest occupied (HOMO) and lowest unoccupied molecular orbital (LUMO) energies of the complexes, we can correlate stability, transport factors and bioavailability to liganding. This can be thoroughly tested using the aforementioned electrochemical analysis and vibrational spectroscopy techniques. So far modeling has been done using DMol3 software from Accelrys: initial results for simple Pu(VI) compounds will be expanded upon by inclusion of a solvation shell. More extensive modeling can be accomplished through the P.I.'s interaction with Jim Kubicki at Pennsylvania State University.

Overall, these results point to a broad-based and exciting effort to understand transformation of TRU complexes exposed to redox environments such as microbial activity. Efforts toward all the objectives described here will continue and are likely to produce a novel paradigm for analytical modeling which has applicability to a broader range of activities of DOE concern.

**DELIVERABLES:** Conference presentation and abstract:

Charlotte W. Eng, Gary P. Halada, A. J. Francis and Cleveland J. Dodge, Environmental effects on aqueous uranium-organic complexes: A structural and electrochemical study, abstract GEOC 5, The 228th ACS National Meeting, Philadelphia, PA, August 22-26, 2004